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TITLE: TGF β 1 Regulation of Matrix Metalloproteinase-9 in Human Prostate Cancer Metastasis

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13. Abstract (Maximum 200 Words) (abstract should contain no proprietary or confidential information) The purpose of this project is to identify and test portions of the 3 prime untranslated region of the Matrix Metalloproteinase-9 mRNA responsible for TGF β -induced increases in mRNA stability. This is to be accomplished by generating constructs of the 3'UTR and comparing them with the entire MMP-9 coding sequence in transfection experiments. This past year I obtained a modified SOW, in which part of our second task was changed to allow us to use our MMP-9 3'UTR deletion constructs in <i>in vitro</i> invasion assays and then measure MMP-9 expression and inhibition of invasion as end points. Western blot analyses and zymography using one cell line suggest a portion of the 3'UTR, when overexpressed, can reduce TGF β -stimulation of MMP-9. Northern blots were being conducted. A serious problem has arisen with this research project. I have recently obtained a faculty position elsewhere. Upon learning of my departure, NDSU has given me only restricted access to my office and laboratory. Notebook and all reagents pertinent to this project have been seized. As I write this report, I am not able to communicate with the student who worked on this project, nor have I been allowed access to her data, notebook or reagents.		
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Introduction:

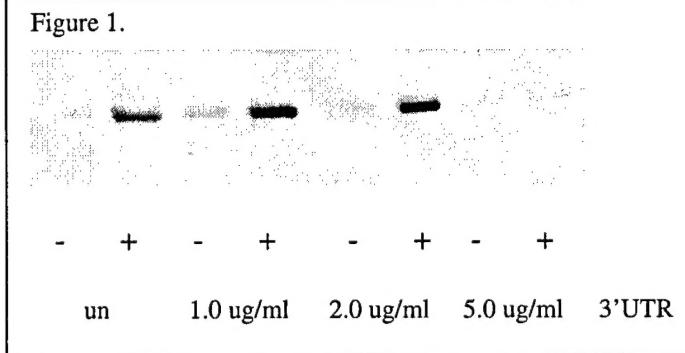
The purpose of this project is to identify and test portions of the 3 prime untranslated region of the Matrix Metalloproteinase-9 mRNA responsible for TGF β -induced increases in mRNA stability which results in a growth factor-induced increase in MMP-9 protein expression. This is to be accomplished by generating constructs of the 3'UTR and comparing them with the entire MMP-9 coding sequence in transfection experiments to identify discrete regions of the MMP-9 3'UTR which mediate TGF β 1 induction of increased MMP-9 mRNA half-life. We will analyze oligonucleotide sequences of the MMP-9 transcript for TGF β 1-regulated protein-binding and ultimately characterize the binding proteins as regulators of mRNA stability. We will attempt to use portions of the 3'UTR to inhibit the increased expression of MMP-9 mRNA and protein and *in vitro* invasion.

Body:

Positive outcomes this past year: This past year, we modified our Statement of Work. In our original Statement of Work, we outlined 3 Specific aims encompassing 5 tasks. For our second task, we originally proposed to transfer regions of the MMP-9 transcript onto a portion of the bacterial neomycin resistance gene (neo) to determine if the 3'UTR of MMP-9 could induce TGF β -1-enhanced stability in the neo gene. Although this task would provide informative biochemical data, we believed a more biological approach geared towards a therapeutic endpoint to be a priority. We proposed to use our MMP-9 3'UTR deletion constructs *in vitro* and to then measure TGF- β 1 induction of MMP-9 through northern blots, western blots and zymograms. In addition, we would test the ability of 3'UTR transfectants to reduce Matrigel invasion.

Figure 1 shows a western blot depicting conditioned medium from transfected Tsu-Pr1 cells. The untreated lane (un) shows cells transfected with an empty plasmid, minus (-) or plus (+) TGF β 1. There is a robust increase in MMP-9 expression. In the presence of 1.0 or 2.0 ug/ml 3'UTR-containing plasmid, TGF β 1 still induces MMP-9; however in the presence of 5.0 ug/ml TGF β 1, the response to TGF β 1 is not apparent.

Figure 1.



Negative outcomes and difficulties: This past year, my research efforts have been seriously impeded. My laboratory progress has been repeatedly slowed or stopped because of building construction which has been ongoing since June 2001. This 12-month grant period between June 29 through July 9, my laboratory was shut down completely and without warning. Because of my cell cultures, I pleaded with both my Dean and with the construction foreman to gain

emergency access to cells yet this was refused. This sudden closure resulted in the loss of numerous cell lines used in this study. Our research infrastructure has become less and less supportive: our College's only liquid scintillation counter was given away and we now lack a working high speed centrifuge. I had to work without a laminar flow in my tissue culture hood from May 2 until August 19th because of purchasing delays and because our College administration refused to install a new damper. Cell line contamination has therefore been common place. My laboratory was re-keyed without warning or issuance of new keys to myself and my workers in August. A different cell culture facility which I designed to have positive air flow was built with negative air flow allowing construction dust into the facility. Because of these and other limitations on my research, I sought and obtained a more research intense faculty position. I am relocating my program to Louisiana State University in Baton Rouge at the College of Veterinary Medicine effective July 1. However, upon learning of my departure from a graduate student working on this project, NDSU has reacted by essentially halted my research effort. I have been given 2 hours a day of restricted access to my office and laboratory and my other workers have 4 hours per day. Some notebooks and all reagents in the laboratory as of April 25 pertinent to this project have been seized. As I write this report, I am not able to communicate with the student who worked on this project, nor have I been allowed access to her notebooks. I believe it is important for me to point out that no patentable data has been obtained, and no end-stage reagents have been generated. Indeed because of the extensive delays I have faced here, our data remains promising but preliminary. Yet, the university attorney and my Dean have assumed the project is of great value and they fear my continuance of the present project at another institution with existing reagents. As should be apparent from this and from my past reports, these reagents could be duplicated in a research intense environment in weeks to months; therefore I have no need or desire to take the constructs built here at NDSU which have been removed from my laboratory anyway. I have written to my College Dean and requested that he immediately return all data and results applicable to this project in order for me to fulfill my legal obligation to the DoD in writing this report. This request has not been granted. I apologize for not being able to communicate more data in this report.

Key research accomplishments:

- Transfections of 100 base pair sections of the MMP-9 3' UTR demonstrate some reduction in the ability of TGF- β 1 to stimulate MMP-9 secreted activity (zymography) and protein levels (western blots of conditioned media) in one cell line.

Reportable outcomes:

Presentations:

"Proteases In Prostate Cancer Metastasis", Invited Seminar, Louisiana State University, Baton Rouge, LA, March 6, 2003.

"Proteases In Prostate Cancer Metastasis", Invited Seminar, Oklahoma State University, Stillwater, OK, February 11, 2003.

Employment or research opportunities applied for and/or received based on experience/training supported by this award:

Associate Professor faculty position, LSU Baton Rouge, College of Veterinary Medicine.

Conclusions: This grant could not be accomplished within the environment existing at the College of Pharmacy at NDSU. It can be accomplished at a research intense university environment. Complete research experiments must be verified by analyses of the mRNA half life; however, protein data suggest that TGF β 1 functions by inducing protective elements that inhibit degradation since competitive inhibition of these protective elements with overexpressed portions of the UTR reduces some of the ability of TGF β 1 to induce MMP-9.

To further address this hypothesis, this grant must be conducted in a research intense environment.

“So what” section

The delineation of specific sections of the MMP-9 3'UTR which can decrease MMP-9 protein when overexpressed, could lead to the development of a specific method of targeting growth-factor induction of MMP-9 by prostate cancer cells. This could be a great improvement over the use of chemical MMP inhibitors, which are a class of compounds that have struggled to find success in human trials.

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